Eur J Vasc Endovasc Surg (2009) **37**, 728–734

REVIEW

Functional Imaging of Atherosclerosis to Advance Vascular Biology

N. Sakalihasan [a,*], **J.B. Michel** [b]

[a] *Chirurgie cardiovasculaire, CHU de Liège, 4000 Liège Sart-Tilman, Belgium*
[b] *Cardiovascular remodelling, INSERM Unit 698, 46, rue Henri Huchard, CHU Bichat, Claude Bernard, 75018 Paris, France*

Submitted 5 December 2008; accepted 30 December 2008
Available online 15 February 2009

KEYWORDS
Abdominal aortic aneurysms;
Atherosclerosis;
PET scanning;
MRI

Abstract Preliminary events leading to the rupture of atherosclerotic plaques or aneurysmal wall expansion undoubtedly are linked to altered and increased metabolism of cells in the vascular wall. To allow *in vivo* identification of this local activity, imaging techniques such as positron emission tomography (PET) and contrast ultrasonography may be used. However, the use of complementary multimodal imaging methods, such as computed tomography (CT), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), etc., can inform about other processes, including vascular wall calcification, haemosiderin deposits, apoptosis and accumulation of activated platelets in the arterial wall. Such techniques may be used as an adjunct in following the evolution of the disease, as well as having crucial roles as molecular and cellular probes of arterial disease. Therefore, functional imaging techniques may be able to help us take more reliable decisions on the need for medical or surgical treatment of arterial disease.

Conventional imaging of the vascular tree using ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI) and contrast angiography gives us anatomical and morphological information about localised vascular disease. However, recently available imaging techniques can provide a molecular and cellular assessment of atherothrombosis at the level of the arterial wall.

Knowledge of the biological activities associated with the progression of atherosclerosis towards clinical expression in humans has evolved considerably in the last years. In this article, atherosclerosis will include occlusive forms (stenosis) and dilating forms (abdominal aortic aneurysm, AAA). The common role of haemorrhage and thrombosis, and the angiogenesis-driven inflammatory response in vulnerable plaques, offers new opportunities for functional imaging of biological activities in atherosclerosis. Since specific molecular mechanisms (matrix synthesis and proteolysis) are also implicated in the development of aneurysmal and occlusive disease, other modalities for

* Corresponding author. Tel.: +32 4 366 71 63; fax: +32 4 366 71 64.
E-mail address: nsaka@chu.ulg.ac.be (N. Sakalihasan).

1078-5884/$34
doi:10.1016/j.ejvs.2008.12.024

following the evolution of these processes might also be possible.

Better understanding of the pathogenesis and natural history of these processes, with the goal of improving the treatment of atherothrombosis and aortic aneurysm, should be an important goal for physicians. Therefore, the goal is to identify functional imaging methods to inform about both biological mechanisms and disease prognosis, particularly with respect to the prediction of impending rupture of aneurysms and vulnerable plaques.

Haemorrhage and Thrombosis: a Common Pathway in Vulnerable Plaques and AAA

Compared to the role of lipids and immuno-inflammation[1] in the initiation and progression of atheromatous diseases, the biological determinants of plaque or wall rupture are less well documented in humans. The involvement of repeated intra-plaque haemorrhages in the evolution of atherothrombotic lesions was proposed as early as 1938.[2] This important topic has been recently revisited by Kolodgie et al.[3] They found an association between unesterified cholesterol accumulation and specific red blood cell (RBC)-antigen expression. Platelet membranes also participate in cholesterol accumulation.[4] The intra-plaque haemorrhage, leading to the formation of a necrotic core, includes all blood components: RBCs, leukocytes (70% neutrophils), platelets[5] and plasma proteins, all of which are retained by encapsulation of the core between the cap and the media.

AAA is another frequent form of atherothrombosis.[6] AAA is the archetypal human model of proteolytic injury of the arterial wall leading to rupture.[7] Intra-luminal thrombus (ILT) activities are polarised: initiated at the luminal interface by circulating blood, these activities are progressively conveyed towards the aneurysm wall. Luminal platelet activation and fibrin formation bring zymogens from the blood towards the aneurysm wall, thus participating in the extracellular matrix degradation.[8] On the abluminal pole, ILT is completely degraded and resembles the necrotic core of vulnerable plaque. Therefore, AAAs represent an accessible spatio-temporal pathophysiological model of human atherothrombosis, allowing a study of the associations between biological activities and clinical expression.

Inflammation and Angiogenesis: a Common Response to Vulnerable Plaque and AAA

As early as 1938, in parallel with the observations of intra-plaque haemorrhage in vulnerable plaques, Paterson[9] reported the development of neo-capillaries in complicated plaques and their involvement in sub-intimal haemorrhage. Such observations led Barger et al.[10] to propose that neo-angiogenesis influences the progression of atherosclerosis. In their remarkable study, using post-mortem micro-angiography and corresponding histological examination of coronary arteries, Kumamoto et al.[11] demonstrated that neo-vascularisation of the atherosclerotic plaque originated mainly from the adventitia and rarely from the lumen. In humans, these neo-capillaries develop mainly in the shoulder of the complicated plaque, at the interface between the core, the cap and the media. These neo-capillaries would allow diffusion of plasma-borne molecules and leukocyte diapedesis. Indeed, the density of intimal neo-capillaries correlated with the extent of core formation, haemosiderin deposits, haemorrhage and inflammatory infiltrates, suggesting that centripetal angiogenesis is linked to the evolution of atherothrombosis. Similar data on neo-vascularisation have been reported in human carotid plaques[12–14] and the aorta,[15] correlating in all cases with plaque evolution.

Although monocytic cell migration from blood to the intima is thought to be mainly aimed at phagocytosis and initiation of the early atheromatous process, inflammatory infiltrates are present in the shoulder region of the lesion core and in the adventitia adjacent to complicated plaques. A mononuclear cell infiltration associated with atheromatous plaques was reported by Gerlis, in 1956, in coronary arteries[16] and by Schwartz and Mitchell, in 1962.[17]

In AAA, proteolytic injury results in initiation of inflammation, oedema and lymphoid neo-genesis in the adventitia. Inflammatory cell retention and lymphoid neo-genesis are linked to capillary development in the adventitia in AAA.[18] Neo-angiogenesis in the outer part of the AAA was described more than 10 years ago.[19] The authors showed strong spatial correlations between neo-capillaries, degradation of elastin and the extent of the inflammatory infiltrate in the outer aortic wall.[19] However, in contrast to vulnerable plaques or occlusive thrombus,[20] neo-vessels do not colonise the media and the luminal thrombus in AAA, probably because of a local excess of proteolytic activities.

Therefore, imaging techniques enabling visualisation of both biological processes involved in the processes of haemorrhage and thrombus formation and angiogenetic inflammation would be highly desirable.

X-Ray and CT Scan

Calcification is a common feature in all forms of atherosclerosis and is easily identifiable by X-ray. Calcium phosphate precipitation and retention in soft tissue are linked to cell death and membrane-particle formation and to trans-differentiation of smooth muscle cells. In AAA, calcifications are usually localised in the medial layer, defining not only the external side of the aneurysmal dilatation, but also occasionally within the ILT. These medial calcifications potentially are linked to smooth muscle death. In addition, interfaces between calcified and normal tissue create areas of high stress and strain, which could increase extracellular matrix sensitivity to breakdown. Calcifications are frequently present in plaques, creating 'artefacts' for ultrasound and MRI. They could be an important determinant of plaque evolution by proliferating within the lumen and creating stenosis and 'hot spots' for thrombosis. Calcifications are particularly frequent in the iliac and femoral arteries. Automatic quantification of calcified areas has long been suggested as a useful prognostic tool in coronary artery disease,[21] but has not been used widely in peripheral arterial disease.

Crescent sign in AAA. The crescent sign, caused by contrast entering the intra-luminal thrombus and high attenuation within ILT detected by CT scan, was reported some time ago.[22] The crescent sign corresponds to 'liquefaction' or bleeding into the intra-luminal thrombus.[23] Since the bleeding comes directly from the lumen, this sign is best appreciated in the arterial phase of the scan. Subsequently, this morphological sign has been correlated with an impending risk of aneurysmal rupture.[24–26] This imaging of bleeding into the ILT has been recently reviewed and showed a high association between AAA rupture and the presence of a crescent sign.[27] The crescent sign can also be demonstrated and analysed with MRI.

Magnetic Resonance Imaging (MRI)

In both, haemorrhagic areas and thrombus, the erythrocytic component releases paramagnetic iron from haem, which can be identified based on the T1-weighted shortening effect, and by the signal loss associated with iron deposition when T_2-weighted (T_2W) gradient-echo or T_2W spin-echo sequences are used.[28] The presence of the T_2W negative signal is associated with the presence of cells capable of phagocytosing haemosiderin and storing it as intracellular iron—ferritin complexes. These are primarily macrophages and polynuclear neutrophils. Other accessory phagocytic cells, such as smooth muscle cells,[29] and other biological processes associated with binding and concentrating iron cannot be excluded. Nevertheless, signal loss and negative contrast obtained using conventional T_2/T_2W may lack sensitivity and specificity, because of the low relaxivity of iron (sensitivity), and can be confused (specificity) with the signal loss caused by other sources, such as partial-volume artefact.

Therefore, imaging of iron phagocytic activity could be enhanced by iron-particle contrast agents, such small paramagnetic iron oxide (SPIO) or other formulations of iron particles. These exogenously injected iron particles should be phagocytosed, thereby enhancing the T_2W sequences.[30] Recently, more specific and sensitive sequence analyses, such as gradient-echo acquisition for superparamagnetic particles/susceptibility (GRASP), have been proposed for positive imaging of tissue iron retention.[31]

Histological observations of intra-plaque haemorrhages compared with MRI of human carotid atheroma allowed identification of intra-plaque haemorrhage[32,33] as the main determinant of plaque evolution.[34]

Scintigraphy

Single photon emission computed tomography (SPECT) is a tomographic imaging technique using gamma rays. It is very similar to conventional planar nuclear medicine imaging using a gamma camera. However, it is able to provide true three-dimensional information. This information is typically presented as cross-sectional slices through the patient, but can be freely reformatted or manipulated as required. Imaging of atheromatous plaques has traditionally centred on assessing the degree of luminal narrowing; indeed, myocardial perfusion imaging is a routinely used tool for exploring such functional haemodynamic consequences.

More recently, it has become clear that it is of utmost importance to identify vulnerable atherosclerotic plaques responsible for the majority of life-threatening syndromes. Molecular imaging using nuclear medicine techniques has the potential to characterise the activity of atheromas.

Annexin V specifically binds, with nanomolar affinity, to phosphatidyl serine (PS), which is exposed on the surface of activated platelets[35] and apoptotic cells.[36] Therefore, radiolabelled ^{99m}Tc-annexin-V has been used for *in vivo* scintigraphic imaging of both apoptotic cells in animals and humans[37] and acute or chronic platelet-rich thrombi in animals.[38,39] In a recent study, we have shown the ability of ^{99m}Tc-annexin-V to assess the renewal activity of chronic aseptic intra-luminal thrombi, at the interface between circulating blood and thrombus, in an *in vivo* experimental model of AAA, and *ex vivo* in human ILT.[37] In another study, we extended this result to experimental endocarditis and demonstrated that ^{99m}Tc-Annexin-V can be used for visualising thrombotic vegetations — an observation that was confirmed in a case report of endocarditis in a human patient.[40] Moreover, ^{99m}Tc-Annexin-V scintigraphy also could help to localise peripheral emboli, and thus could probably be used to evaluate therapeutic efficacy.

Since exposure of PS is one of the mediators of platelet-activation-induced fibrin formation, ^{99m}Tc-Annexin-V imaging could provide noninvasive functional information on the renewal rate of haemo-thrombus, which is constantly in contact with flowing blood. Unfortunately, despite the tremendous potential of ^{99m}Tc-Annexin-V imaging in cardiovascular diseases, this radiotracer is not yet commercially available for clinical use.

In addition to annexin V, lactadherin is capable of binding to PS of any origin, whether from platelets and/or cell apoptosis.[41,42] Lactadherin can also bind PS as well as α(v) β(5) and (3) integrin.[43] The potential of this ligand for molecular imaging has not yet been explored.

Another peptidic (13 amino acids, cyclo-(D-Tyr-Apc-Gly-Asp-Cys)-Gly-Gly-Cys(Acm)-Gly-Cys(Acm)-Gly-Gly-Cys-NH_2) ligand of GPIIb/IIIa has been developed, ^{99m}Tc-Apcitide[44] (Acutect®, Diatide, USA) for visualising biological activity associated with thrombi.[45,46] This radiotracer is mainly proposed for exploring deep venous thrombosis and pulmonary embolism.[47]

Since proteolytic activity plays a major role in the rupture of the arterial wall associated with plaque and/or AAA, imaging this multifactorial biological process, possibly including the fibrinolytic pathway, could be an interesting challenge. Aprotinin is a purified bovine lung protein with powerful affinity for plasmin. It is used as a therapeutic agent in human disseminated intravascular coagulation (Trasylol). Aprotinin radionuclide (^{99m}Tc-aprotinin) already has been used in humans for imaging amyloid deposits[48] to which plasmin binds.[49] This provides the future possibility of imaging plasmin activity *in vivo*.

Positron Emission Tomography (PET)

PET imaging was developed in the mid-1970s; like any nuclear medicine imaging technique, it is based on the detection of photons emitted by the patient after administration of a radio-labelled tracer. Several physical

characteristics of PET constitute a major advantage over monophotonic scintigraphy. Most importantly, the tracers are labelled with positron-emitting radionuclides. The two photons resulting from the disintegration of the positron are emitted in opposite directions (i.e., at 180° from each other) and recorded in coincidence by the detectors surrounding the subject. A detailed description of the specific technical and methodological features of PET imaging is obviously beyond the scope of this article and may be found in Phelps.[50] In short, PET imaging increases the count rate (i.e., the number of photons that are detected) and improves the spatial resolution, that is, lesion detectability. In addition, the images can be fully corrected, in particular for attenuation, which allows for an accurate and reproducible quantitation of tracer distribution.

Depending on the radiotracer, a wide variety of physiological and pathological processes can be studied at the molecular level using this technique. However, in routine clinical practice, vast majority of PET studies are performed using 18-F-fluorodeoxyglucose (FDG), which reflects glucose uptake and metabolism resulting from cellular activity.

FDG is a glucose analogue, transported into cells using glucose transporters. Once inside the cells, FDG is phosphorylated to FDG-6-phosphate, which is not a substrate for the enzymes of the glycolytic chain, and hence FDG-6-phosphate accumulates within the cell. FDG-PET recognises increased metabolic activity and is mainly used for cancer imaging. Indeed, cell glucose metabolism is significantly increased in most types of cancer[51] due to increased expression of membrane transporters, increased hexokinase activity or both. Nevertheless, FDG uptake is not specific for tumours. Increased uptake is observed in many non-neoplastic physiological and pathological conditions.[52] Usually, the level of FDG uptake by inflammatory cells in the resting state is low in comparison with tumour cells. However, when activated, these cells may show significant increases in glucose uptake and metabolism. This has been evaluated in various experimental settings, including skin transplantation, turpentine-induced inflammation, concavalin-A activation of T lymphocytes in bacterial abscesses or in B lymphocytes after viral infection. The lack of specificity for tumours provides a powerful tool for using PET to evaluate inflammatory and infectious diseases as well as during the monitoring of vascular graft infection.[53–55] It should be noted, however, that FDG uptake is often seen in the arterial wall, in the absence of any known inflammatory vascular disease. Yun et al. evaluated two series of patients who underwent PET imaging for oncological or other indications. They found that the rate of positive vessel uptake approached 50% and increased with age.[56] They also showed that hypercholesterolaemia and age were the only parameters correlated with the presence of such uptake, among all major risk factors for atherosclerosis.[57]

With the advent of modern PET/CT, the procedure has been considerably shortened and simplified. Usually, patients are asked to fast for 6 h prior to injecting FDG, which is of particular importance when investigating inflammatory processes, as glucose loading significantly decreases glucose transporter expression (and FDG uptake) in inflammatory lesions.[58] Modern hybrid scanners are coupled with CT for attenuation correction and anatomical mapping. Attenuation correction is usually performed using data from continuous, enhanced, low-dose body CT from the skull base to the thighs. Intravenous contrast enhancement can be used with limited effects on attenuation correction and uptake quantification in order to provide additional information on the thrombus, surrounding tissues and vessels.

Wu et al.[59] demonstrated the uptake of ^{18}F-FDG in the unstable atherosclerotic carotid plaque and correlated this with levels of circulating matrix metalloproteinase 1 (MMP-1). Other workers have shown the high uptake of FDG into vulnerable atherosclerotic plaque[60] and the reduction of carotid FDG uptake after statin therapy. This suggests that PET imaging might have clinical utility in monitoring atherosclerotic arterial disease.[61]

Using autoradiographic techniques, Rudd et al. showed increased tracer accumulation in the regions of the plaque with the highest density of macrophages.[62] Indeed, enhanced uptake has been reported in various inflammatory diseases involving the large vessels. Giant cell arteritis and Takayasu arteritis both show significantly increased glucose metabolism in the wall of the affected arteries (i.e., aorta, subclavian arteries or carotid arteries).[54] Furthermore, FDG uptake has been found in large arteries in the presence of active atheromatous plaques.[56,57]

In a pilot study, Sakalihasan et al. observed an association between 18-FDG uptake by the aneurysm wall and rapid expansion of the aneurysm in some cases.[63] Indeed, five of the nine operations on patients with positive PET imaging were performed on an urgent basis. In the 16 PET-negative patients, aneurysmal repair was delayed for the convenience of the patient from one to several months. None of these patients developed aneurysm-related symptoms in the interval.

FDG uptake in the aneurysm wall reflects the presence of increased metabolic activity, probably associated with a high density of inflammatory cells (macrophages, lymphocytes, etc.) in the adventitia, as previously described.[64] These preliminary observations have been confirmed recently in a study by Reeps et al.,[65] where they observed a correlation between increased FDG uptake and patients with a very high macrophage activity and symptomatic AAA. However, in agreement with earlier reports,[63] Reeps et al. failed to find a correlation between the maximum standard uptake value (SUV) and maximum cross-sectional infra-renal AAA diameter.[65] These studies could suggest a possible correlation between increased FDG uptake by the aneurysm wall and inflammatory cell biology leading to rupture.

Therefore, PET scanning with FDG uptake offers a new tool for exploring adventitial immuno-inflammatory responses in atherosclerosis and AAA.

New Ligands and Contrast Ultrasonography

The biology of human atherothrombotic processes is complex, and the possible molecular and cellular targets are numerous. Several biological processes could be targeted by molecular imaging including: lipid retention, platelet activation and aggregation, coagulation and fibrin formation, proteolysis (including MMP activities[66]), serine proteases

Table 1 Functional imaging of pathobiological processes in the vasculature

Imaging modality	Pathobiological processes imaged
CT scanning	Calcification
PET	Highly metabolically active cells, particularly macrophages in the arterial wall
MRI	Haemosiderin deposits in the arterial wall
SPECT	Apoptosis, activated platelets
Contrast ultrasonography	Neo-vascularisation and cell receptors

(plasmin, elastase, etc.), free extracellular haemoglobin, etc. Here there are opportunities for contrast-enhanced ultrasonography.[67] Protein or peptide ligands (including antibodies) can be attached to the micro-bubbles used for contrast to target endothelial epitopes, leukocyte adhesion molecules and angiogenesis receptors.[67]

Conclusion

Multimodal imaging, associated with different complementary methods such as CT scan and PET, MRI and scintigraphy and contrast ultrasonography, will improve the definition of the atherothrombotic lesion and its pathobiology. The biological process imaged by these different imaging techniques are summarised in Table 1. The use of several complementary methods associated with morphological, functional and molecular analysis could allow a better definition of the prognosis of the lesion.

Conflict of Interest

The authors have no conflict of interest.

Funding

The authors have no funding.

This study was partially supported by a grant from the University of Liege ''Credit de l'impulsion''. The cardiovascular surgery department in Liège and Inserm unit 698 in Paris are supported by the European Union integrated project ''Fighting Aneurysmal Disease'' (FAD, http://www.fighting-aneurysm.org/).

References

1 Tedgui A, Mallat Z. Cytokines in atherosclerosis: pathogenic and regulatory pathways. *Physiol Rev* Apr 2006;**86**(2):515—81.
2 Wartman WB. Occlusion of the coronary arteries by hemorrhage into their walls. *Am Heart J* 1938;**5**:459—70.
3 Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, et al. Intraplaque hemorrhage and progression of coronary atheroma. *N Engl J Med* Dec 11 2003;**349**(24):2316—25.
4 Chandler AB, Hand RA. Phagocytized platelets: a source of lipids in human thrombi and atherosclerotic plaques. *Science* Sep 29 1961;**134**:946—7.
5 Gawaz M, Langer H, May AE. Platelets in inflammation and atherogenesis. *J Clin Invest* Dec 2005;**115**(12):3378—84.
6 Sakalihasan N, Limet R, Defawe OD. Abdominal aortic aneurysm. *Lancet* Apr 30—May 6 2005;**365**(9470):1577—89.
7 Michel JB. Contrasting outcomes of atheroma evolution: intimal accumulation versus medial destruction. *Arterioscler Thromb Vasc Biol* 2001;**21**(9):1389—92.
8 Fontaine V, Touat Z, Mtairag el M, Vranckx R, Louedec L, Houard X, et al. Role of leukocyte elastase in preventing cellular re-colonization of the mural thrombus. *Am J Pathol* Jun 2004;**164**(6):2077—87.
9 Paterson JC. Capillary rupture with intimal haemorrhage as a causative factor in coronary thrombosis. *Arch Pathol* 1938;**25**:474—87.
10 Barger AC, Beeuwkes 3rd R, Lainey LL, Silverman KJ. Hypothesis: vasa vasorum and neovascularization of human coronary arteries. A possible role in the pathophysiology of atherosclerosis. *N Engl J Med* Jan 19 1984;**310**(3):175—7.
11 Kumamoto M, Nakashima Y, Sueishi K. Intimal neovascularization in human coronary atherosclerosis: its origin and pathophysiological significance. *Hum Pathol* Apr 1995;**26**(4):450—6.
12 Milei J, Parodi JC, Alonso GF, Barone A, Grana D, Matturri L. Carotid rupture and intraplaque hemorrhage: immunophenotype and role of cells involved. *Am Heart J* Dec 1998;**136**(6):1096—105.
13 McCarthy MJ, Loftus IM, Thompson MM, Jones L, London NJ, Bell PR, et al. Angiogenesis and the atherosclerotic carotid plaque: an association between symptomatology and plaque morphology. *J Vasc Surg* Aug 1999;**30**(2):261—8.
14 Mofidi R, Crotty TB, McCarthy P, Sheehan SJ, Mehigan D, Keaveny TV. Association between plaque instability, angiogenesis and symptomatic carotid occlusive disease. *Br J Surg* Jul 2001;**88**(7):945—50.
15 Moreno PR, Purushothaman KR, Fuster V, Echeverri D, Truszczynka H, Sharma SK, et al. Plaque neovascularization is increased in ruptured atherosclerotic lesions of human aorta: implications for plaque vulnerability. *Circulation* Oct 5 2004;**110**(14):2032—8.
16 Gerlis LM. The significance of adventitial infiltrations in coronary atherosclerosis. *Br Heart J* Apr 1956;**18**(2):166—72.
17 Schwartz CJ, Mitchell JR. Cellular infiltration of the human arterial adventitia associated with atheromatous plaques. *Circulation* Jul 1962;**26**:73—8.
18 Satta J, Soini Y, Mosorin M, Juvonen T. Angiogenesis is associated with mononuclear inflammatory cells in abdominal aortic aneurysms. *Ann Chir Gynaecol* 1998;**87**(1):40—2.
19 Holmes DR, Liao S, Parks WC, Thompson RW. Medial neovascularization in abdominal aortic aneurysms: a histopathologic marker of aneurysmal degeneration with pathophysiologic implications. *J Vasc Surg* May 1995;**21**(5):761—71 [discussion 771—62].
20 Thompson MM, Jones L, Nasim A, Sayers RD, Bell PR. Angiogenesis in abdominal aortic aneurysms. *Eur J Vasc Endovasc Surg* May 1996;**11**(4):464—9.
21 Kaufmann RB, Peyser PA, Sheedy PF, Rumberger JA, Schwarts RS. Quantification of coronary artery calcium by electron beam computed tomography for determination of severity of angiographic coronary artery disease in younger patients. *J Am Coll Cardiol* 1995;**25**:626—32.
22 King PS, Cooperberg PL, Madigan SM. The anechoic crescent in abdominal aortic aneurysms: not a sign of dissection. *AJR Am J Roentgenol* Feb 1986;**146**(2):345—8.
23 Gasser TC, Gorgulu G, Folkesson M, Swedenborg J. Failure properties of intraluminal thrombus in abdominal aortic aneurysm under static and pulsating mechanical loads. *J Vasc Surg* Jul 2008;**48**(1):179—88.
24 Siegel CL, Cohan RH, Korobkin M, Alpern MB, Courneya DL, Leder RA. Abdominal aortic aneurysm morphology: CT features

in patients with ruptured and nonruptured aneurysms. *AJR Am J Roentgenol* Nov 1994;**163**(5):1123–9.
25 Mehard WB, Heiken JP, Sicard GA. High-attenuating crescent in abdominal aortic aneurysm wall at CT: a sign of acute or impending rupture. *Radiology* Aug 1994;**192**(2):359–62.
26 Arita T, Matsunaga N, Takano K, Nagaoka S, Nakamura H, Katayama S, et al. Abdominal aortic aneurysm: rupture associated with the high-attenuating crescent sign. *Radiology* Sep 1997;**204**(3):765–8.
27 Roy J, Labruto F, Beckman MO, Danielson J, Johansson G, Swedenborg J. Bleeding into the intraluminal thrombus in abdominal aortic aneurysms is associated with rupture. *J Vasc Surg* Nov 2008;**48**(5):1108–13.
28 Haque TL, Miki Y, Kanagaki M, Takahashi T, Yamamoto A, Konishi J, et al. MR contrast of ferritin and hemosiderin in the brain: comparison among gradient-echo, conventional spin-echo and fast spin-echo sequences. *Eur J Radiol* Dec 2003; **48**(3):230–6.
29 Deux JF, Dai J, Riviere C, Gazeau F, Méric P, Gillet B, et al. Aortic aneurysms in a rat model: in vivo MR imaging of endovascular cell therapy. *Radiology* Jan 2008;**246**(1):185–92.
30 Hyafil F, Laissy JP, Mazighi M, Tchétché D, Louedec L, Adle-Biassette H, et al. Ferumoxtran-10-enhanced MRI of the hypercholesterolemic rabbit aorta: relationship between signal loss and macrophage infiltration. *Arterioscler Thromb Vasc Biol* Jan 2006;**26**(1):176–81.
31 Mani V, Briley-Saebo KC, Itskovich VV, Samber DD, Fayad ZA. Gradient echo acquisition for superparamagnetic particles with positive contrast (GRASP): sequence characterization in membrane and glass superparamagnetic iron oxide phantoms at 1.5 T and 3 T. *Magn Reson Med* Jan 2006;**55**(1):126–35.
32 Kampschulte A, Ferguson MS, Kerwin WS, Polissar NL, Chu B, Saam T, et al. Differentiation of intraplaque versus juxtaluminal hemorrhage/thrombus in advanced human carotid atherosclerotic lesions by in vivo magnetic resonance imaging. *Circulation* Nov 16 2004;**110**(20):3239–44.
33 Leiner T, Gerretsen S, Botnar R, Lutgens E, Cappendijk V, Kooi E, van Engelshoven J. Magnetic resonance imaging of atherosclerosis. *Eur Radiol* Jun 2005;**15**(6):1087–99.
34 Takaya N, Yuan C, Chu B, Sam T, Polissar NL, Jarvik GP, et al. Presence of intraplaque hemorrhage stimulates progression of carotid atherosclerotic plaques: a high-resolution magnetic resonance imaging study. *Circulation* May 31 2005;**111**(21): 2768–75.
35 Thiagarajan P, Tait JF. Binding of annexin V/placental anticoagulant protein I to platelets. Evidence for phosphatidylserine exposure in the procoagulant response of activated platelets. *J Biol Chem* Oct 15 1990;**265**(29):17420–3.
36 Thiagarajan P, Tait JF. Collagen-induced exposure of anionic phospholipid in platelets and platelet-derived microparticles. *J Biol Chem* Dec 25 1991;**266**(36):24302–7.
37 Sarda-Mantel L, Coutard M, Rouzet F, Raguin O, Vrigneaud JM, Hervatin F, et al. 99mTc-annexin-V functional imaging of luminal thrombus activity in abdominal aortic aneurysms. *Arterioscler Thromb Vasc Biol* Sep 2006;**26**(9):2153–9.
38 Stratton JR, Dewhurst TA, Kasina S, Reno JM, Cerqueira MD, Baskin DG, et al. Selective uptake of radiolabeled annexin V on acute porcine left atrial thrombi. *Circulation* Nov 15 1995; **92**(10):3113–21.
39 Tait JF, Cerqueira MD, Dewhurst TA, Fujikawa K, Ritchie JL, Stratton JR. Evaluation of annexin V as a platelet-directed thrombus targeting agent. *Thromb Res* Sep 1 1994;**75**(5): 491–501.
40 Kietselaer BL, Narula J, Hofstra L. The Annexin code: revealing endocarditis. *Eur Heart J* 2007 Apr;**28**(8):948.
41 Shi J, Gilbert GE. Lactadherin inhibits enzyme complexes of blood coagulation by competing for phospholipid-binding sites. *Blood* Apr 1 2003;**101**(7):2628–36.
42 Shi J, Heegaard CW, Rasmussen JT, Gilbert GE. Lactadherin binds selectively to membranes containing phosphatidyl-L-serine and increased curvature. *Biochim Biophys Acta* Nov 17 2004;**1667**(1):82–90.
43 Andersen MH, Graversen H, Fedosov SN, Petersen TE, Rasmussen JT. Functional analyses of two cellular binding domains of bovine lactadherin. *Biochemistry* May 23 2000; **39**(20):6200–6.
44 Francesconi LC, Zheng Y, Bartis J, Blumenstein M, Costello C, De Rosch MA. Preparation and characterization of [99TcO] apcitide: a technetium labeled peptide. *Inorg Chem* May 3 2004; **43**(9):2867–75.
45 DeHoyos MJ, Rubal BJ, Bradley MY. Detection of acute atrial thrombus in a porcine model with atrial fibrillation with tc 99m-apcitide. *Invest Radiol* Apr 2004;**39**(4):197–201.
46 Carretta RF. Scintigraphic imaging of lower-extremity acute venous thrombosis. *Adv Ther* Nov–Dec 1998;**15**(6):315–22.
47 Dunzinger A, Hafner F, Schaffler G, Piswanger-Soelkner LC, Brodmann M, Lipp RW. (99m)Tc-apcitide scintigraphy in patients with clinically suspected deep venous thrombosis and pulmonary embolism. *Eur J Nucl Med Mol Imaging* Nov 2008;**35**(11): 2082–7.
48 Sojan SM, Smyth DR, Tsopelas C, Mudge D, Collins PJ, Chatterton BE. Pharmacokinetics and normal scintigraphic appearance of 99mTc aprotinin. *Nucl Med Commun* Jun 2005; **26**(6):535–9.
49 Schaadt BK, Hendel HW, Gimsing P, Jønsson V, Pedersen H, Hesse B. 99mTc-aprotinin scintigraphy in amyloidosis. *J Nucl Med* Feb 2003;**44**(2):177–83.
50 Phelps ME. PET: the merging of biology and imaging into molecular imaging. *J Nucl Med* 2000;**41**:661–81.
51 Hustinx R, Bénard F, Alavi A. Whole-body imaging in the management of patients with cancer. *Semin Nucl Med* 2002;**32**: 35–46.
52 Strauss LG. Fluorine-18 deoxyglucose and false-positive results: a major problem in the diagnostics of oncological patients. *Eur J Nucl Med* 1996;**23**:1409–15.
53 Zhuang H, Alavi A. 18-fluorodeoxyglucose positron emission tomographic imaging in the detection and monitoring of infection and inflammation. *Semin Nucl Med* 2002;**32**:47–59.
54 Belhocine T, Blockmans D, Hustinx R, Vandevivere J, Mortelmans L. Imaging of large vessel vasculitis with (18)FDG PET: illusion or reality? A critical review of the literature data. *Eur J Nucl Med Mol Imaging* 2003;**30**:1305–13.
55 Wassélius J, Malmstedt J, Kalin B, Larsson S, Sundin A, Hedin U, et al. High ^{18}F-FDG uptake in synthetic aortic vascular grafts on PET/CT in symptomatic and asymptomatic patients. *J Nucl Med* 2008;**49**:1601–5.
56 Yun M, Jang S, Cucchiara A, Newberg A, Alavi A. 18F FDG uptake in the large arteries: a correlation study with the atherogenic risk factors. *Semin Nucl Med* 2002;**32**:70–6.
57 Yun M, Yeh D, Araujo LI, Jang S, Newberg A, Alavi A. F-18 FDG uptake in the large arteries: a new observation. *Clin Nucl Med* 2001 Apr;**26**(4):314–9.
58 Zhao S, Kuge Y, Tsukamoto E, Mochizuki T, Kato T, Hikosaka K, et al. Fluorodeoxyglucose uptake and glucose transporter expression in experimental inflammatory lesions and malignant tumours: effects of insulin and glucose loading. *Nucl Med Commun* 2002;**23**:545–50.
59 Wu Y-W, Kao H-L, Chen M-F, Lee B-C, Tseng WY, Jeng JS, et al. Characterization of plaques using ^{18}F-FDG PET/CT in patients with carotid atherosclerosis and correlation with matrix metalloproteinase-1. *J Nucl Med* 2007;**48**:227–33.
60 Tawakol A, Migrino RQ, Bashian GG, Bedri S, Vermylen D, Cury RC, et al. In vivo ^{18}F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. *J Am Coll Cardiol* 2006;**48**: 1818–24.

61 Tahara N, Kai H, Ishibashi M, Nakaura H, Kaida H, Baba K, et al. Simvastatin attenuates plaque inflammation. *J Am Coll Cardiol* 2006;**48**:1825—31.
62 Rudd JH, Warburton EA, Fryer TD, Jones HA, Clark JC, Antoun N, Johnström P, et al. Imaging atherosclerotic plaque inflammation with [18F]-fluorodeoxyglucose positron emission tomography. *Circulation* 2002;**105**:2708—11.
63 Sakalihasan N, Van Damme H, Gomez P, Rigo P, Lapiere CM, Nusgens B, Limet R. Positron emission tomography (PET) evaluation of abdominal aortic aneurysm (AAA). *Eur J Vasc Endovasc Surg* 2002;**23**:431—6.
64 Defawe OD, Hustinx R, Defraigne JO, Limet R, Sakalihasan N. Distribution of F-18 fluorodeoxyglucose (F-18 FDG) in abdominal aortic aneurysm: high accumulation in macrophages seen on PET imaging and immunohistology. *Clin Nucl Med* 2005 May; **30**(5):340—1.
65 Reeps C, Essler M, Pelisek J, Seidl S, Eckstein HH, Krause BJ. Increased 18F-fluorodeoxy glucose uptake in abdominal aortic aneurysms in positron emission/computed tomography is associated with inflammation, aortic wall instability, and acute symptoms. *J Vasc Surg* 2008;**48**(2): 417—23.
66 Lancelot E, Amirbekian V, Brigger I, Raynaud JS, Ballet S, David C, et al. Evaluation of matrix metalloproteinases in atherosclerosis using a novel noninvasive imaging approach. *Arterioscler Thromb Vasc Biol* Mar 2008;**28**(3):425—32.
67 Villanueva FS. Molecular imaging of cardiovascular disease. *J Nucl Cardiol* 2008;**15**:576—86.